99

31

218

THE GERMICIDAL ACTIVITY OF EMANATIONS FROM IRRADIATED OILS

426-248

(Robert S. Harris, John W.M. Bunker, and Nicholas a Milas)

Research Division of the Department of Biology and Public Health and the Department of Organic Chemistry Research, Mass. Inst. of Technology.

See Ind. Chem Eng. Oct. 1932 p. 1181-1183

Presented before the Division of Medicinal Chemistry, Am. Chem. Soc., Sept. 1, 1931 at Buffalo, New York.

****

In 1930 a report came from Germany that irradiated oils had been used with marked success in clearing up suppurative processes when applied in salve form. Furthermore, it was reported that if these oils were applied with aluminum or tin foil the clearing up of infections was enhanced. Reid, who reported these results, thought that these effects were due to emanations of light from the oils, that ~~the~~ the use of the foil caused an indirect radiation to be formed, and that this radiation was the active substance.

We are in agreement with others that irradiated oils do not give off secondary radiations of light and therefore we tried to find the active cause of the above phenomenon. In preliminary experiments five cubic centimeter amounts of cod liver oil were placed in uncovered Petri dishes and irradiated under a quartz-mercury vapor, horizontal, 6 inch arc at a distance of 18 inches for one hour without stirring. A measured amount of a virulent culture of S. aureus was smeard on the surface of nutrient agar in another series of Petri dishes which were then inverted above the samples of irradiated oil. The ~~distanc~~ distance between the bacterial culture and the irradiated oil was 2 centimeters. Each pair of plates was held in this position by a strip of adhesive tape around the periphery. After this incubation at 37°C. for 24 hours there was no growth on the agar inverted above the irradiated oils, but abundant growth in those plates above the unirradiated oils and in the controls without oil.

105 A

To prove the contention that the effective substance is not an amanation of light a sheet of quartz 4 millimeters thick was placed between the oil and the agar culture in several pairs of Petri dishes. There was no inhibition of growth. The quartz plate was opaque to the germicidal substance. We therefore conclude it was not a secondary radiation, of light.

To prove this same point in another way the irradiated oil was poured in a dish which was closed except for a small opening in the center of the top. A culture of S. aureus was placed above this dish as before. The whole surface of the agar was free from growth after incubation. If the germicidal substance had been a radiation, then that portion directly above the opening would have been without growth while the outer portion of the agar would habe shown normal growth.

This same point was demonstrated in still a third way. Irradiated oil in a small dish was loosely covered with an oversized top and this container enclosed in a glass chamber with inverted agar culture. Any emanations from the oils traveled only through a devious path. After incubation there was no growth on the agar. The germicidal substance could therefore not have been a radiation.

Having found that the germicidal substance is gaseous, the next step was to identify it. Various dilutions of formaldehyde in water were tested as to germicidal action. It was found that vapors from dilutions less that 1:1000 were germicidal to S. aureus.

A five cc. sample of irradiated cod liver oils was placed in a test tube. A smaller test tube containing aldehyde test solution was suspended through a cork stopper into the first tube which was itself stoppered. The smaller tube had a hole several centimeters above the level of the test solution which allowed the interchange of vapors between the two tubes. After 24 hours at 37°C. the test solution showed no aldehyde. This would indicate that the germicidal substance in the case of cod liver oil is not aldehyde.

In discussing what other substance might be the cause of this action we decided to test for peroxidic - oxygen contained in the oils and given off by them. The peroxidic- oxygen of each of 20oils ~~and~~ was determined before and after irradiation for one hour. Although it was found that the peroxidic-oxygen per gram of oil was usually increased by irradiation there was no complete correlation between the peroxidic-oxygen content, or its increase, and the germicidal activity of the oils. In certain cases the peroxidic-oxygen of oils whose vapors had no germicidal activity at all, even after irradiation for four hours, was several times that of other oils which were ghighly germicidal after only fifteen minutes' irradiation. This can be explained by assuming that those oils which are high in peroxidic-oxygen, but do not give off germicidal emanations may, have peroxidic-oxygen compounds which are stable.

In another test nutrient agar was mixed with acidified soluble starch-potassium iodide solution. This agar mixture was inverted over irradiated oil. If peroxidic - oxygen were given off by the oils then it should react with the agar mixture to produce blue coloration. The greated the absorption of the peroxidic-oxygen from the air between the oil and the agar misture the greater should be the intensity of coloration of the agar mixture, over a period of time. If peroxidic- oxygen is the germicidal substance, then a tabulation of the oils according to depth of coloration at the end of a given period of time should correlate with the tabulation of the same oils according to their germicidal properties. This was found to be the case with a few exceptions.

In a further test acidified starch-potassium iodide solution was placed in a test tube setup as previously discussed. Results showed that the depth of the color of the test solution at the end of 24 hours at 37°C. was almost directly porportional to the germicidal activity of the oil vapors.

Five cubic centimeters of each of four typical oils were placed in a series of preparation jars and irradiated for different periods of time. In the center of each of these jars a smaller preparation jar containing potassium iodide test solution was placed. The larger jar was covered and allowed to stand for 24 hours at ~~37°C.~~ 25°C. At the end of this time the peroxidic-oxygen which had been absorbed by the test solution was titrated against .001 normal sodium thiosulfate. It was found that all those oils which were germicidal showed a peroxidic-oxygen titration above 3.0×10^{-6}, under the conditions of the experiment. This represents the absorption time of a gaseous emanation from these oils.

For all of these reasons we conclude that certain oils, after irradiation with ultraviolet light give off a gaseous emanation which prevents the growth of bacterial surface cultures on agar exposed to these emanations. One such emanation has been shown to be some form of peroxidic-oxygen. Further identification studies are in progress.

The germicidal activity of a number of oils against nine typical pathogenic and non-pathogenic cultures of bacteria, one yeast and one mold has been determined. All these organisms failed to grow in the presence of the oil vapors and upon removal of the oil, followed by reincubation, no growth appeard, proving death rather than inhibition.

Cod liver oil vapors are germicidal after the oil has been irradiated 15 minutes under the above conditions of irradiation. Samples of cod liver oil were irradiated under a series of ultra-violet light filters for periods of time adjusted so that the total intensity under each filter at wave length 3130 A. was the same as that under the bare arc, Preliminary results show that the active portion of the ultraviolet spectrum in producing this bactericidal effect of oil vapors is in this region. These studies are being developed.

Cod liver and cottonseed oils were irradiated one hour then degassed with a high vacuum. This degassin process removed dissolved gases. Exposure of S. aureus to these oils showed that the oils still had germicidal activity. This proves that the germicidal substance which leaves the oil is being formed continually. Oils have been stored sixmonths after irradiation with slight diminution in the germicidal activity of their vapors.

Vogel (Trans. Roy. Soc. S. Africa 18,295, 1930) claimed that the cause of the Russell effect of oils was not due to the formation of ozone or hydrogen peroxide, buth that one of the osidation products, normal butyric acid, is capable of producing the Russell effect. We therefore determined the germicidal effect of dilutions of butyric acid in mineral oil, and found that even 1% is not germicidal. The effective substance can hardly be butyric acid. Since all the oils which were germicidal had high iodine numbers, the unsaturation of these oils was determined. There was no absolute correlation between the iodine number of the oil and the germicidal activity of its vapor. The same is true of acid numbers.

A sample of linoleic acid (Kahlbaum c.p.) , unirradiated, proved to be germicidal to S. aureus and on test for volatile peroxidic -oxygen gave a positive test. The following oils, unirradited, also showed this activity: highfatty acid fish oil, seal and tunafish oil.

Conclusions:

1. The vapors of certain oils exhibit germicidal activity.
2. The irradiation of some oils by U.V. light increases this activity.
3. The portion of the spectrum most active in this respect is in the region of 3130 A.
4. The germicidal substance is not a radiation, but is gaseous.
5. It has been shown by correlations that this germicidal action is presumably due to volatile substances containing peroxidic-oxygen.
6. There is no correlation between this activity and iodine and acid numbers.
7. The positive results obtained from degassed oils shows that the peroxidic-oxygen is continually produced over a period of time.
8. An explanation is suggested for the increased therapeutic action of irradiated oils in contact with metal foil by the expected catalytic ation of metals in the production in the oil of substances containg peroxidic-oxygen.